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THERAPEUTIC EFFECT OF *AGERATUM CONYZOÏDES* LINNEE OINTMENT ON BURN AND DIABETIC WOUNDS INDUCED IN WISTAR RATS.

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ABSTRACT

Aim: The purpose of this work was to qualitatively analyze the ointment of the aqueous extract of the leaves of the *Ageratum conyz*oides L. and to evaluate the effects of this ointment on the burn wounds and the wounds of induced diabetics in the wistar rat. **Methods** Conventional experimental methods were used. **Results** : The obtained cream of *Ageratum conyzoides* is brown in colour, slightly bitter, with a ph=5.6 and not irritating to the skin. This ointment used at doses of 4 and 8 g/kg significantly reduces deep wounds and burns of 2nd Degreaser after 8 days with a complete healing on the 18th day compared to controls with gaping wounds. *Ageratum conyzoides* allows the wound reepithelialisation with an average duration of about 8 days. **Conclusion** : This confirms its use in primary health care among African populations in particular.

KEYWORDS: ointment, burn wound, diabetic wound *Ageratum conyzoides* Linnée.

INTRODUCTION

Medicinal plants are endowed with therapeutic properties that are used as the main source of medicines, providing raw material or serving as a model for the synthesis of therapeutic molecules.[1]

Ageratum conyzoides Linné, of the family Asteraceaeis a well-known plant in the Congolese pharmacopoeia for the treatment of several symptoms and diseases including abdominal pain, gastriculcer, wounds, although the applications and types of preparation vary.[2,3,4,5,6] This work focused on assessing the healing potential of the plant on deep wounds such as bedsores and second-degree burn wounds .Indeed, a wound is rupture of the skin barrier, which occurs at aggressions of the type: surgical, burns, radiation, tears, scratches, abrasions and pressures etc.[7] Now a days, we are witnessing an explosion of cases of wounds due to certain chronic pathologies or their complications that lead to localized lesions whose repair and regeneration processes can be brief; the case of superficial and deep wounds.[7] Also, wounds are a frequent reason for consultation in emergency departments. Because this condition is apparently common place, it can pose complex therapeutic problems.[7] For example, burns, which are very common in developing countries, are a major public health problem because the incidence of serious complications such as infections is high and financial resources are sometimes limited.[8] So, the use of plants by the populations, for centuries is a real palliative in the management of wounds.[9]

MATERIALS AND METHODS

Material

Plant material

The plant material use dis made up of the fresh leaves of *Ageratum conyzoides o*harvested in the northern part of Brazzaville in August 2016 and the identification of the plant was made at the herbarium of the Institut of Natural and exact sciences (IRSEN).

Matériel animal

We used randomized Albino Wistar rats weighting between 200 ± 50 g. These rats were housed in cages at room (in animalary of Superior Normaly school, Marien NGOUABI University) constant temperature under 12/12 night/dark. They hadacess to standard pellet diet and water ad libitum.

Method

Preparation of the aqueous extract

100 g of dry leaf powder from *Ageratum conyzoides* is mixed in 1,500 ml distilled water and boiled at 100°C for 15 minutes. The collected solution is filtered and evaporated in a double boiler at 56°C. The resulting concentrate was used to prepare the ointment.

Preparation of the ointment of the aqueous extract of *Ageratum conyzoides*

4 and 8 g of the dry residue of aqueous extract of *Ageratum conyzoides* were solubilized in 10 ml of Tween 80. The solution obtained was mixed with pure vaseline. The dough was distributed in clean, sterile pots and placed in the refrigerator at 4°C.[10]

Characterization of organoleptic and physico-chemical parameters of *Ageratum conyzoides* ointment

Organoleptic parameters of *Ageratum conyzoides* ointment

They consist of appreciating the taste, smell, irritability and texture of *Ageratum conyzoides* ointment.[11] We have realized the:

Primary skin irritation test

0,4 g of *Ageratum conyzoides* ointment at the respective doses of: 4 and 8 g were applied to the healthy and scarified skin of wistar rats. These dressings are held by compress and tape for 24 to 72 hours. The degree of irritation is evaluated macroscopically, 30 minutes after the dressing is removed.[12]

Eye irritation test

0,5 mg ointment at 4 and 8 g/kg doses was applied to the right eye of each wistar rat in the batches used. These rats are held in a restraint cage before 18 hours before the test. Then, we proceed to the observations with the naked eye, of the conjunctiva to check the coloring of the eye (enanthem), which translates a possible swelling and eyelids (chemosis) checking for possible tingling, the watering of the eye ball soaked with ointment. This was for 1, 24 and 48 hours after.[13]

Physico-chemical analysis of the ointment

The hydrogen potential or pH was measured using a HYDROCHECK pH meter; the dissolved substances (TDS) and conductivity were determined.

Evaluation of healing activity of *Ageratum conyzoides* ointment on induced 3rd degree burn wounds in wistar rats

The healing power is verified by means of a product capable of causing contraction and reepithelialisation of the skin keratinocytes, after a lesion. Five (05) batches of five male and female rats were distributed as follows: batches 1 and 2 (controls), received Dakin® or vaseline, by local application; batches 3, 4 and 5 are treated with the reference product Silver Sulfadiazine 1% + Chitosan hydrochloride 2% (Curapel®) and *Ageratum conyzoides* ointment at 4 and 8 g/kg, respectively. After shaving, third-degree burns were performed at the dorsal level in rats.

Induction of burnwounds

A weight of 50 g /kg and a round contact surface of 2-3 cm in diameter served as a tool for induction of burn wounds. The weight is heated in boiling water at 100°C, until heat equilibrium (reachedin 5 min). After boiling, it is removed from the water, quickly wiped, then applied without pressure, for 40 seconds at the dorsal level of the place of election on the rat previously stabilized by anesthesia with ethyl ether. Wound measurements are taken over night in a planimetric study.[13,14,15]

Treatment of wounds

Two (2) minutes after induction of the burn wounds, the rat lots were each treated with the test product. These products are applied and the wounds are cleaned with sodium hypochloride (Dakin®) daily, once in 24 hours.

Planimetric study

The technique used is that described by (Bhat et al.,2007 cited by Thakur et al., 2011), which consists of assessing the evolution of burn wound healing by digital planimetry.[16] Wounds at regular intervals of four (04) days are photographed, then the evolution of their average areas and the percentage of wound shrinkage are determined. Note that, the period of re-epithelialization of the wound is dependent on the time of the fall of the bedsore and complete closure of the wound.

Evaluation of the healing effect of the *Ageratum conyzoides* ointment on the wounds of rats rendered diabetic

This test consists in making incision wounds on rats made diabetic, in order to evaluate the effect of the product in the treatment of wounds of diabetics. Only a healing and anti-diabeticproductwouldbe protective. Eight (8) batches of five (5) randomized rats wereused, suchthatbatches 1,4 and 5 are controls, non-diabetic (NDNT, Dtvas) and diabeticrenderers, which receive nothing; batches 3,4,7 and 8, non-diabetic (NDT A.c) and diabetic (DT A.c) treated with *A. conyzoides* ointment at 4 and 8 g/kg; batch 6 is treated with the reference product Cicatryl® (Dtcicat).

Induction of Diabetes

We used alloxane monohydrate, which is an inducer of type I hyperglycemic diabetes in humans. The rats in the experiment were fasted 16 hours before and their blood sugar was taken to reassure themselves of their normal state.

A single dose injection of 150 mg/kg body weight (SI) of alloxane monohydrate diluted in a normal 0.9% sterile saline solution was administered in fasting Wistar rats. After this injection, the rats systematically had free access to the diet and a 5% glucose solution in 24 hours. To avoid hypoglycemic shock.[17] After 72h, which follows this induction, the sampling (at the rat caudal vein) and blood glucose measurements are carried out using a portable URIT G28 glucose meter. Only rats with fasting blood sugar above 250 mg/dl were considered diabetic and selected for the study.[18]

Induction of wounds
Rats diagnosed with diabetes before hand, anesthetized with ethylether are placed in prone position, and shavedwith a clipper. They then under go excision in the dorsal region, inducing surgical wounds of about 1cm² in depth, with a scalpel blade.[19] These rats are thenplaced in aseptic conditions, in order to avoid contamination with pathogeni cgerms.

Treatment of wounds
Daily dressings with Dakin® are performed on the wounds of experimental rats and are treated with different products at 0.40 g of ointment once a day. As well as, parameters such as weight, blood sugar and measurement of the average area of wounds, are evaluated.

Weight Collection (Weight, Blood Sugar, Mean Area of Wounds).
The rat weight variation in each lot was measured, one in three (3) days, using a precision scale of 1000 g capacity and 0,1 g sensitivity through out the experimental phase.

Taking of bloodsugar
Blood is collected from the rat's caudal vein and blood glucose was measured using a reactive blood glucose monitor (URIT G28). The evolution of rat glucose in each batch was recorded every three (3) days through out the experimental phase. Also, 1,5 IU/kg MI insulin was administered in rats as a remedy for hyperglycemic complications.

Planimetric study
The lengths and widths of the excision wounds of the different lots of rats were measured using a graduated ruler, every three days until the wounds were completely healed. The measurements of the average areas of the wounds in the different lots and the percentage of shrinkage or contraction of the wounds were determined the mechanism of action of *Ageratum conyzoides* on the healing process.

Determination of Tnfα (TumorNecrosis Factor alpha).
Tnfα, are specific markers of inflammation. The determination of Tnfα is made from a non-invasive sample using the Sandwich ELISA method, which traps an antigen between a manufacturer antibody and a conjugate.

Blood sample from the rat
Blood was collected from the retro-orbital sinus of the eye using a capillary tube in the fasting wistar rat. The EDTA tubes were used to collect the collected blood and were placed in the centrifuge at 3000 rpm for ten (10) minutes. The serum obtained was used to determine Tnfα. The samples were taken three times after the induction of the wounds, that is to say at J_0, J_{12} and J_{18}.

Experimental protocol
The procedure described by the supplier BOSTER antibody and ELISA expert (support@bosterbio.com) is as follows:

1. Preparere agents + Standards + Controls at 37°C;
2. Remove the number of ELISA strips needed for the test;
3. Add 100 µl of Standard, Sample and Control intowells;
4. Add 100 µl of sample thinner to a well corresponding to White;
5. Cover plate with film paper and incubate for 90 minutes at 37°C;
6. Gently vacuum the wells with their liquid (contents) using a washing machine (Appendix 6);
7. Add 100 µl of Biotinylated TNF anti-Rat antibody to each well;
8. Cover and incubate at 37°C for 60 minutes;
9. Wash the plate three (3) times with washpad;
10. Add 100 µ of Avidine Biotine-Peroxydase complex to eachwell, cover and incubate for 30 minutes at 37°C;
11. Wash the plate five (5) times by adding 300 µl of buffer between each wash;
12. Add 90 µl of Color Developer per well, cover plate and incubate for 25 minutes at 37°C in darkness;
13. Add 100 µl of stop solution in each well, blue colors for 4 standards and clear 3 other standards turn yellow automatically;
14. Read the 450 nm optical densities at the Spectrophotometer.

Statistical analysis
The results obtained are expressed as an average more or less standard error (M ESM) and the analysis of the data was made by the Excel software coupled to the Student test. The significance thresholdis set at $p< 0,05$.

RESULTS AND DISCUSSION

Organoleptic control of *Ageratum conyzoides*L ointment
Ageratum conyzoides ointment has a dark brown colour, is homogenous, creamy to the touch, stable and has a pasty consistency. It is less fragrant, with a slightly bitter taste and solubilizes in Tween 20%.

Skin and eye irritation test
The results from these experiments are expressed in Tables I and II. It is noted that, the aqueous extract of this plant at a given dose and depending on the time would cause a slight and temporary irritation in the skin and eye scarified area between 1 and 24 hours, which follow the administration. The irritability index varies with dose and time.

Table I: Evaluation of primary skin irritation.

Lots/dose	Duration (hour)	Erythema and escarre		Edema	
		Escarrified zone	No escarrified zone	Escarrified zone	No escarrified zone
Ointment *A.conyzoides* 4g/kg	1/2	0	0	0	0
	24	0	0	1	0
	72	1	0	0	0
Ointment *A.conyzoides* 8g/kg	1/2	0	0	0	0
	24	1	0	0	0
	72	0	0	0	0

0 = Absence; 1 = Light or veryweak; PomA.c = ointmentde *Ageratum conyzoides* ; n=4.

Table II: Assessment of the index of eye irritation on the conjunctiva.

Lots/dose	Duration (hour)	Assessment of achievement at conjunctiva level		
		Watery	Chémosis	Enanthemes
Ointment *A.conyzoides* 4g/kg	01	03	01	01
	24	0	0	0
	48	0	0	0
Ointment *A.conyzoides* 8g/kg	01	03	01	01
	24	0	0	0
	48	0	0	0

0 = Absence; 1 = Light or veryweak; PomA.c = ointmentof*Ageratum conyzoides* healing process.This compared to the wounds of the witnesses and the untreate dones, which remain gaping until the 28th day. With wound inhibition percentages varying from: 11, 05 to 74%, and from 08,00 to 89,36% for aqueous extract of *Ageratum conyzoides* at 4 to 8 m/kg. And a 79,91% variation for the reference product used (silver sulfadiazine and chitosine hydrochloride). Contrary to 08% and 0% for Vaseline and Non-treated controls.

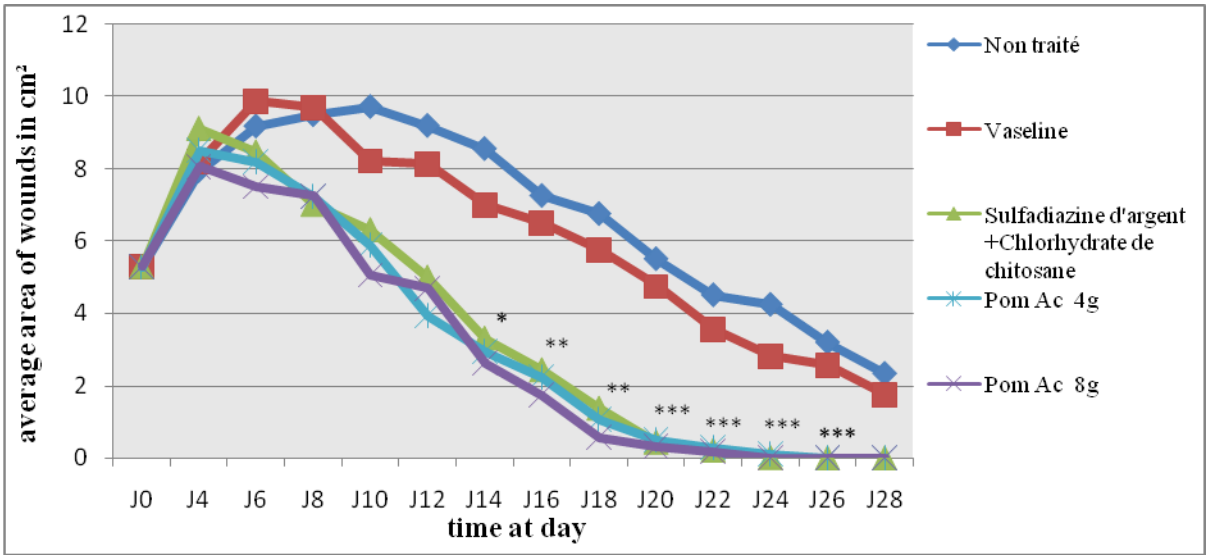


Figure 1: Effect of the *Ageratum conyzoides* ointment on the evolution of the average areas of the wounds of Burns.n=5, p < 0,05; **p < 0,01; ***p < 0,001: significant differences from control lots (Vaseline and untreated). PomA.c : pommade de *Ageratum conyzoides*.

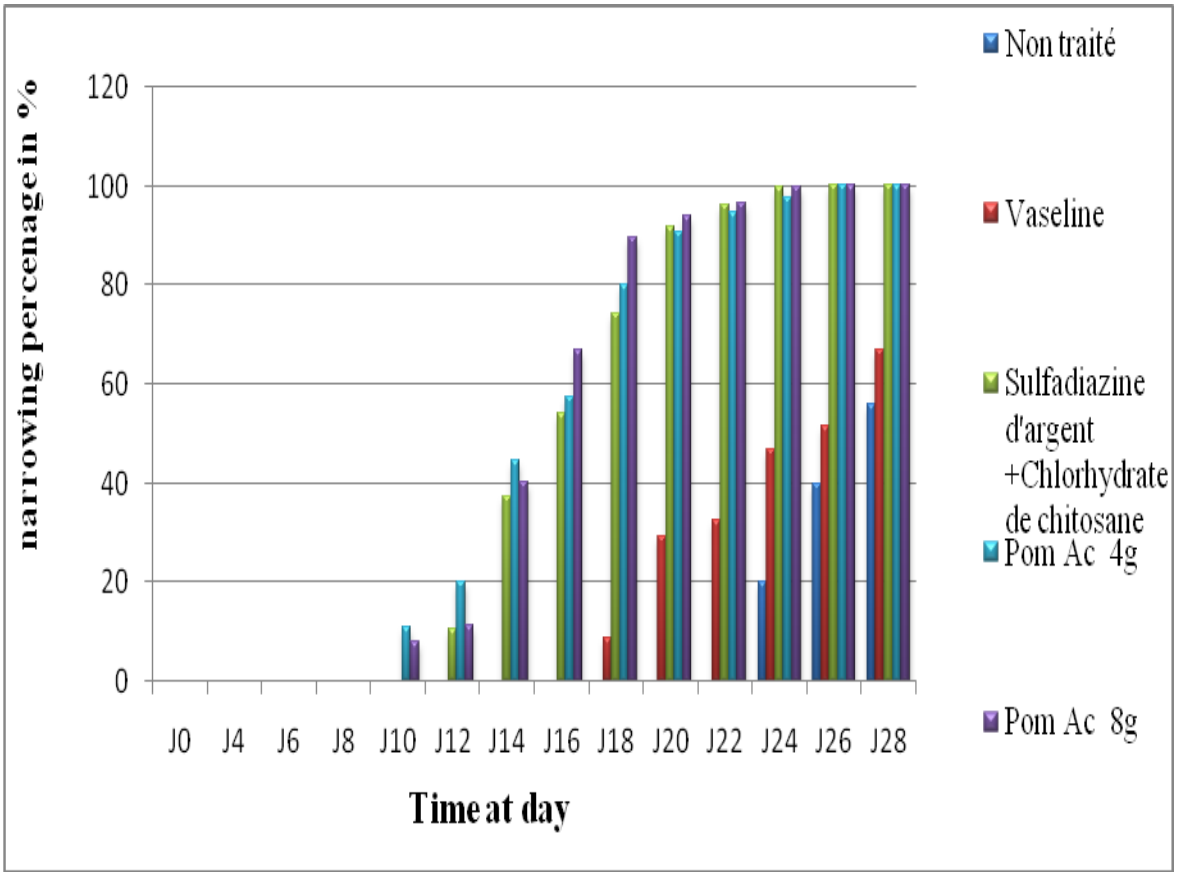


Figure 2: Effect*of Ageratum conyzoides* ointment on the narrowing of burn wounds.

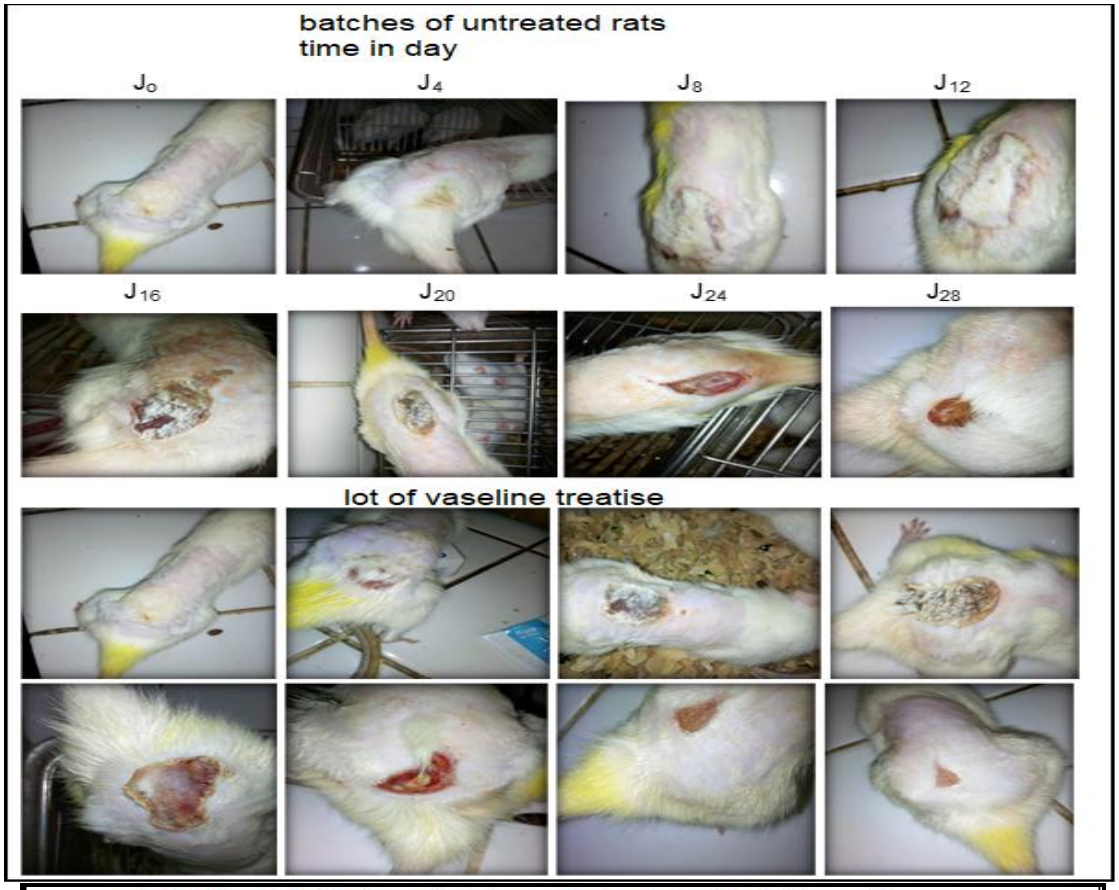
On the other hand, the mean duration of complete reepithelialisation of burnwounds in rats treated with *Ageratum conyzoides* at 4 and 8 g/kg doses is almost short compared to those treated with silver sulfadiazine + chitosine chlorydrate (Table III).

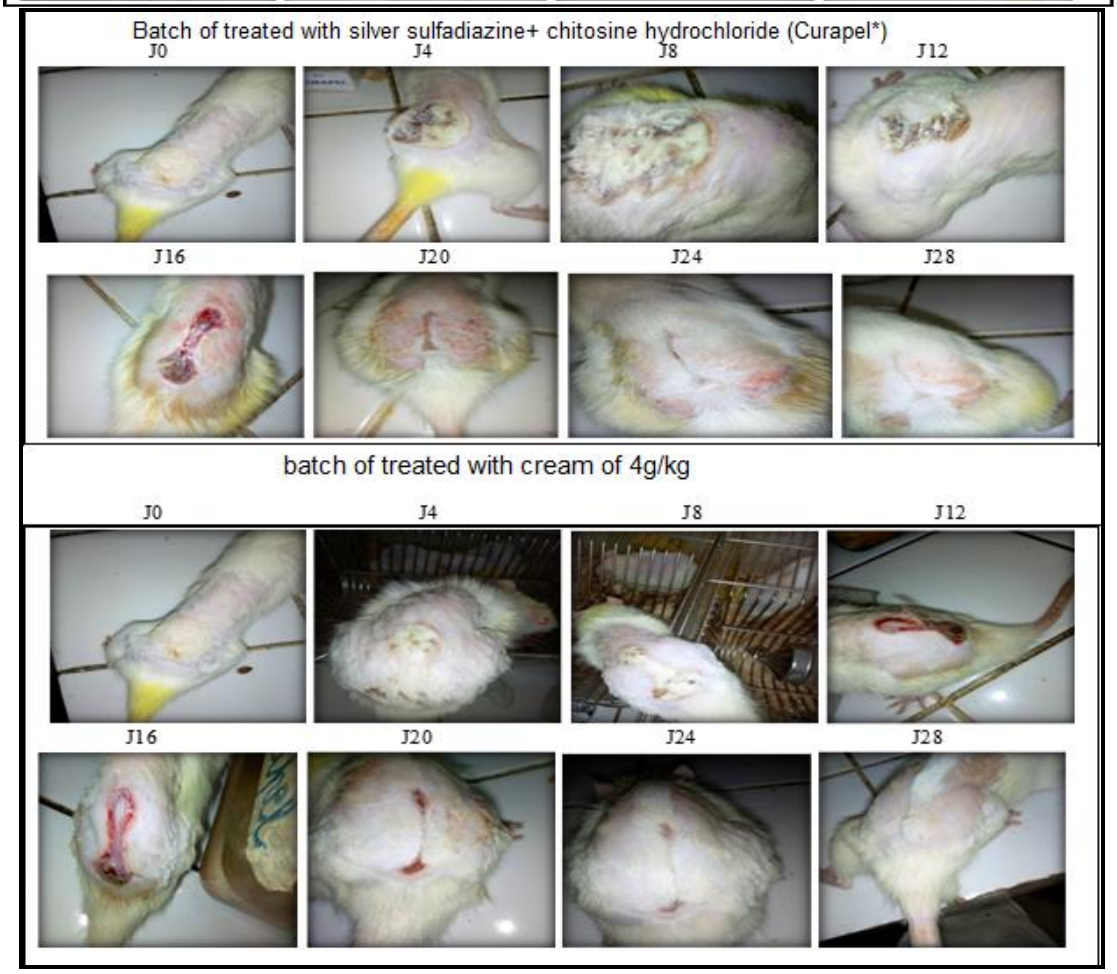
Table III : Mean duration of complete re epithelialisation of batch burns depending on the different products applied (ESM average).

Products/lots	Average duration (day)
Untreated witnesses	22,00 ± 2,32
Witness treated with Vaseline	20,00 ± 1,87
Treated with Silver Sulfadiazine + Chitosine Hydrochloride (Curapel*)	15,00 ± 2,68 **
Treated with *A.conyzoides* Ointment 4g/kg	14,00 ± 1,87 **
Treated with *A.conyzoides* ointment 8g/kg	14,00 ± 1,24 **

**p < 0,01 : significant differences.p < 0,05.

In addition, the effects of the different products tested on the burn wounds induced in the wistar rat are also shown in Figure 3, expressed as images. These observations show that the wounds treated with the ointment of *Ageratum conyzoides* and vaseline showed the appearance of a crust after height (8) days. While, silver sulfadiazine and chistosine chlorydrate (Cuparel®) were treated with eczematous erythema around the wound until the wounds were completely healed.


batches of untreated rats
time in day
Jo
J4
J8
J12
J16
J20
J24
J28
lot of vaseline treatise


Batch of treated with silver sulfadiazine+ chitosine hydrochloride (Curapel*)
J0
J4
J8
J12
J16
J20
J24
J28
batch of treated with cream of 4g/kg
J0
J4
J8
J12
J16
J20
J24
J28

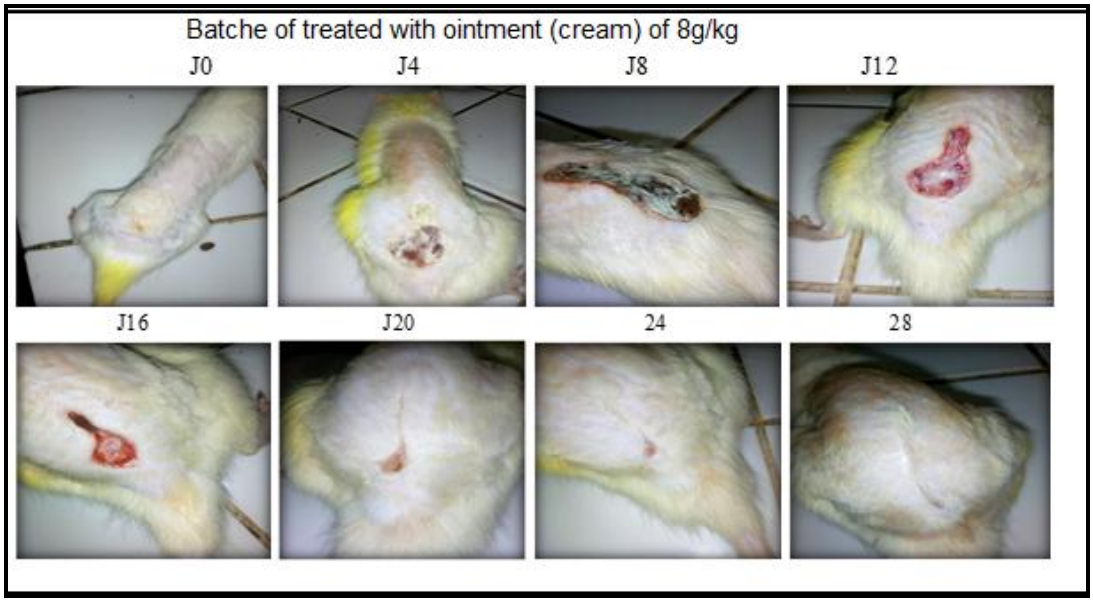


Figure 3: Effect of *Ageratum conyzoides*ointment on burnwounds in Wistar rats.

The weight evolution of the rats tested was one of the parameters thatwe were interested in during our work, as it is closely related to hyperglycemia. The onset of type 1 diabetes is often accompanied by consider able weightloss and weightloss despite heavy foodin take. [20] In fact, insulin deficiency results in a lean weightloss; its diagnosis is therefore easy: the patient loses weight, lean mass and glycemia increases. [21]

Variation in weight of rats in treatment.

Figure 4 shows the histograms of weight changes in non-diabetic rats relative to time (days). There is a non-significant increase in weight in all batches used.

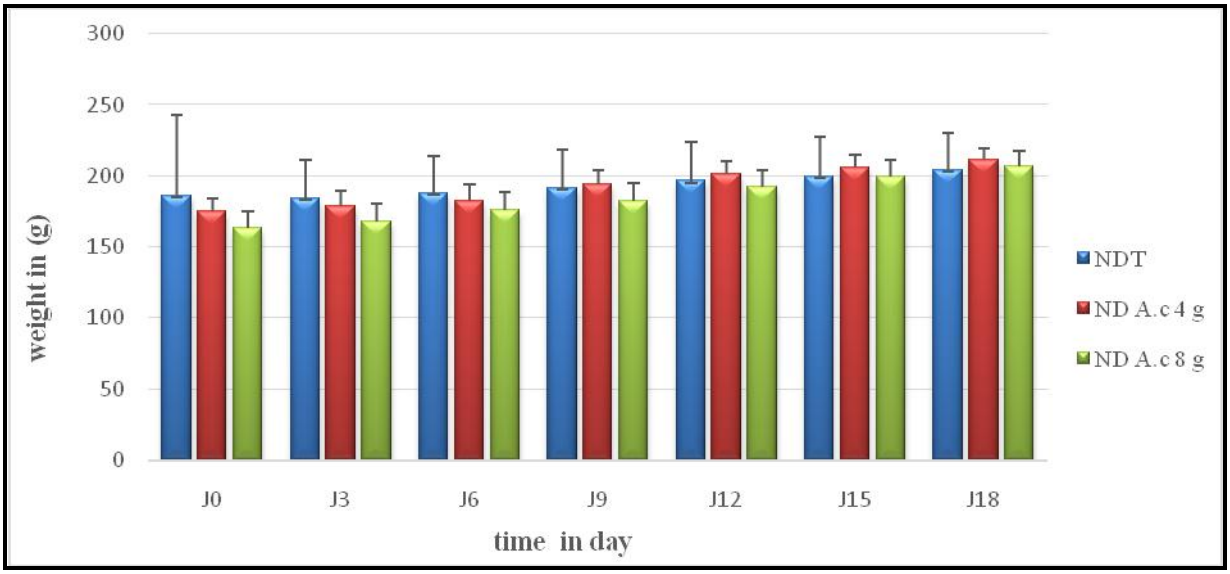


Figure 4: Effect of *Ageratum conyzoides* ointment on the weight evolution (g) of non-diabetic rats. n=4. Note:untreated diabetic (control); Note A.c (4 and 8 g): untreated diabetic treated with *Ageratum conyzoides* ointment à 4 et 8 g/kg.

Weight evolution of diabetic rats

Figure 5 shows the weight (g) of diabetic rats as a function of time (days). There was a significant decrease ($p<0,001$) in weight that occurred six (6) days after the onset of diabetes until the end of the experiment.

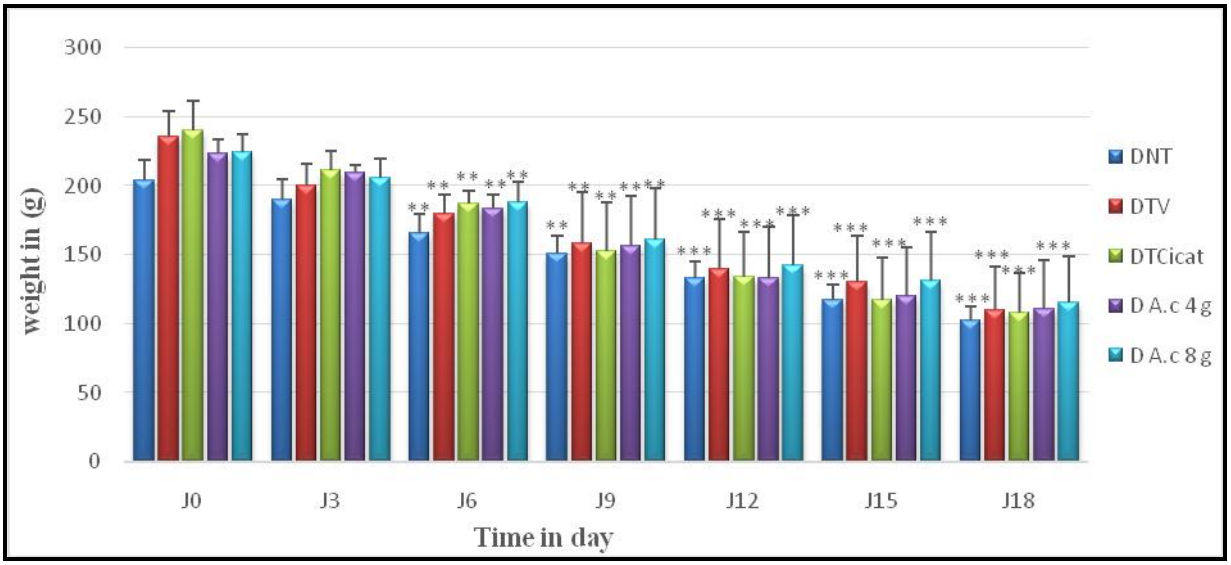


Figure 5: Effect of *Ageratum conyzoides* ointment on the weight evolution (g) of diabetic rats n=4. **p <0.01; ***p <0.001: significant differences from non-diabetic rats. DTN:untreated diabetics; DTV: vaseline-treated diabetics; Dtcicat: Cicatryl® -treated diabetics (reference product); DA.c (4 and 8 g): diabetics treated with*Ageratum conyzoides* ointment at 4 and 8 g/kg.

Blood sugar is the primary parameter that informs about the evolution of the condition of a diabetic subject. For this purpose, the determination of glucose concentration in the rats studied is crucial and indispensable. [22] From where we took blood glucose samples.

Glycemic measurements

Figure 6 shows the results of blood glucose changes in non-diabetic and diabetic rats over the duration of the experiment. It is noted that blood sugar is very high in diabetic rats and is well above the normal threshold (2g/l), while it remains within normal limits (0.9 – 1.2 g/l) in non-diabetic rats.

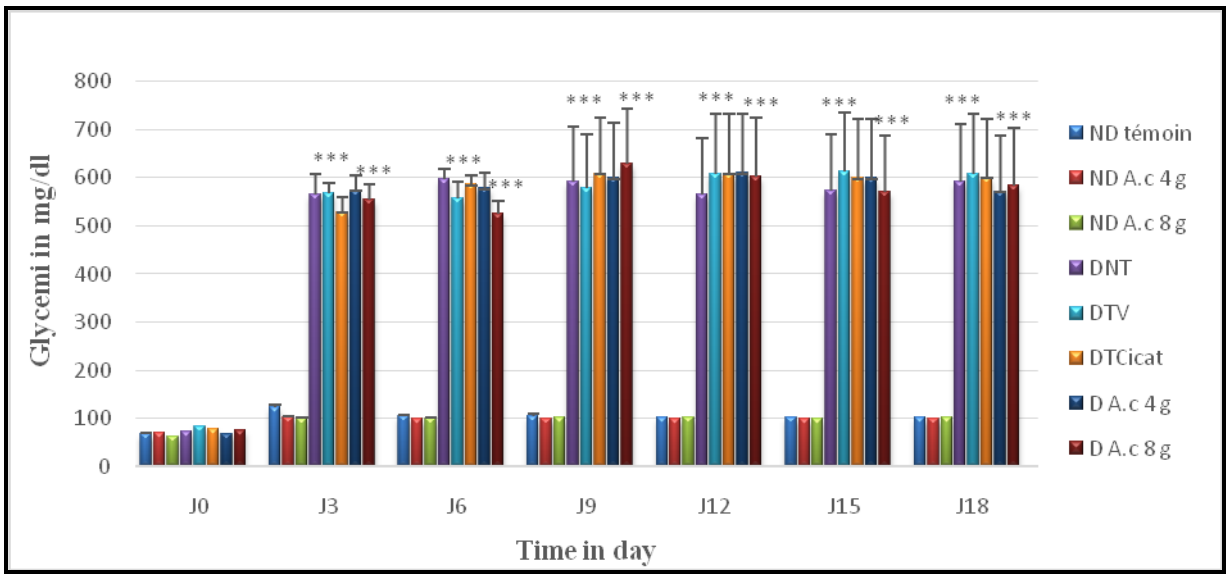


Figure 6 : Effect of *Ageratum conyzoides* ointment on blood glucose (mg/dl) evolution in rats. N = 4. ***p< 0.001: significant difference from non-diabetic rats. Note:untreated diabetic (control); ND A.c (4 and 8 g): diabetic treated with*Ageratum conyzoides* ointment at 4 and 8 g; DTN: untreated diabetic; DTV: diabetic treated with vaseline; Dtcicat: diabetic treated with Cicatryl® (reference product); DA.c (4 and 8 g): diabetics treated with *Ageratum conyzoides* ointment at 4 and 8 g/kg.

Planimetric study of wounds

Figures 7 and 8 show the effects of the ointment of the aqueous extract of *Ageratum conyzoides* on the excision woundsinduced in non-diabetic and diabetic rats. The average duration of reepithelialisation or healing of wounds in non-diabetic rats treated with ointment 4 and

8g/kg is found to be significantly less than that of diabetic rats treated with the same product. The rates of wound contraction in diabetic rats treated with ointment were approximately 33.71% for 4 g/kg and 17.56% for 8 g/kg at J_{18}.

Compared to 100% for non-diabetic rats at 4 and 8 g/kg. For diabetic rats treated with Cicatryl®, there is a significant reduction in wound banks with a contraction rate of 73.87% at J18. However, there was no decrease in the wounds of untreated and vaseline-treated diabetic rats. These wounds remain constant and gaping until J_{18}, while those of untreated non-diabetic rats gradually retract, with a percentage of inhibition of up to 70, 98%.

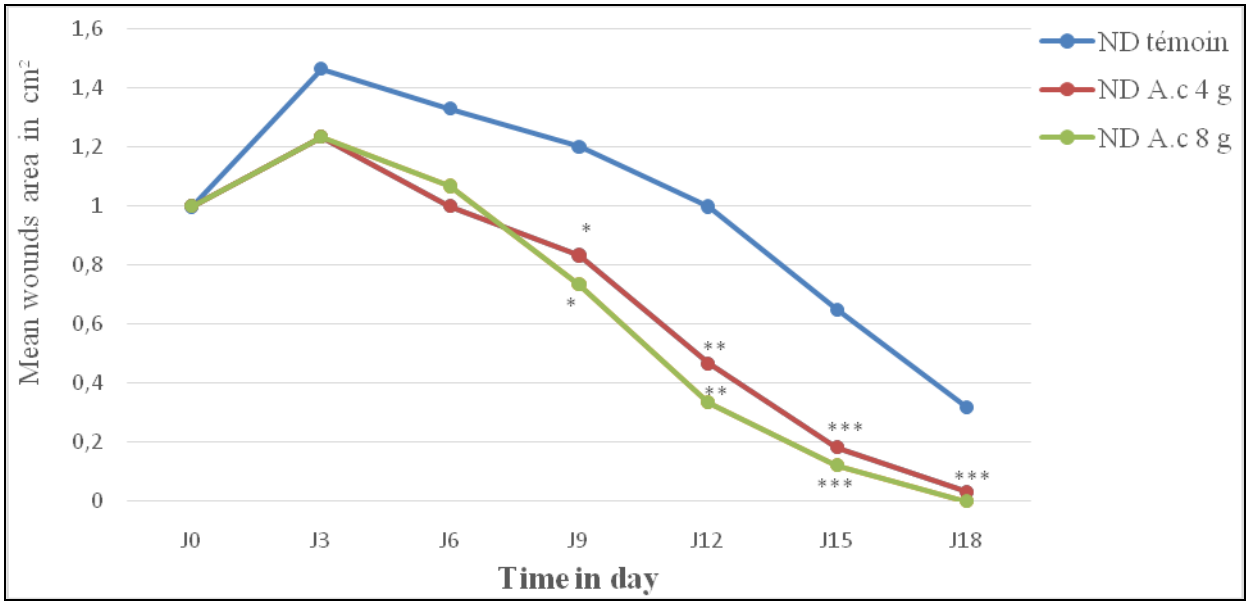


Figure 7: Effect of *Ageratum conyzoides* ointment on the average area (cm²) of the wounds of non-diabetic rats treated with Ageratum conyzoides ointment. n=4, *p<0.05; **p<0.01; ***p<0.001: significant differences from control lots (untreated). Note: not diabetic untreated (control); ND A.c (4 and 8 g/kg): not diabetic treated with *Ageratum conyzoides* ointment at 4 and 8 g/kg.

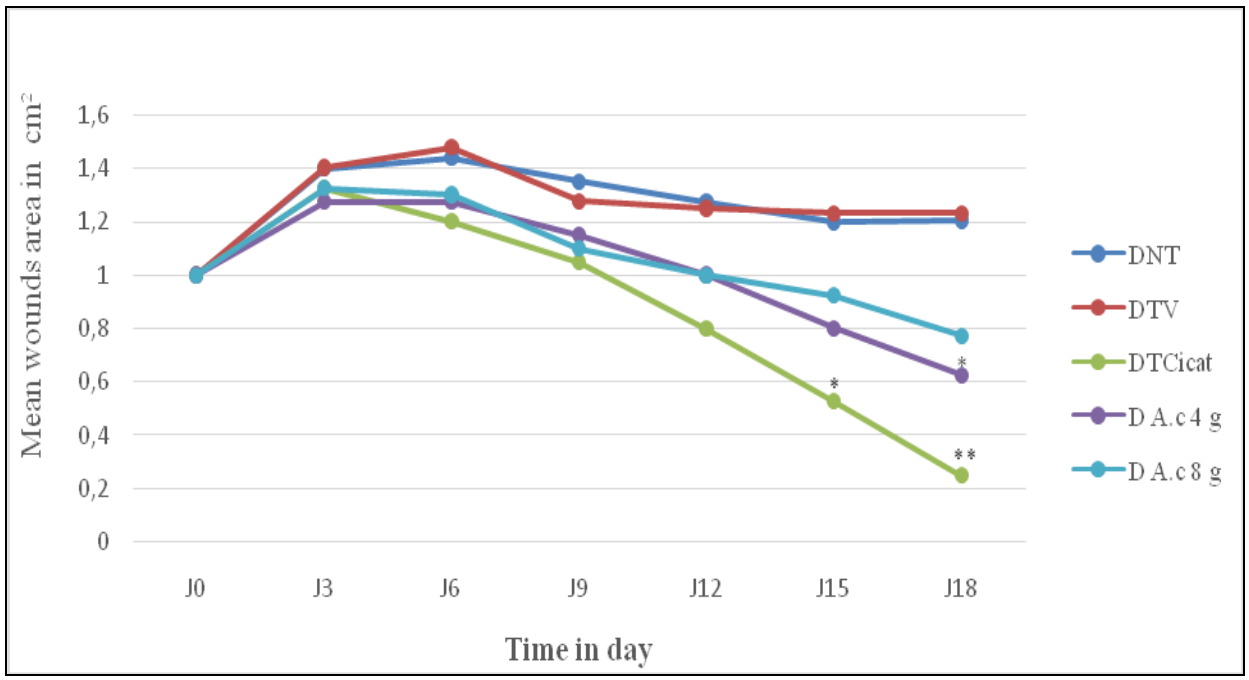


Figure 8: Effect of *Ageratum conyzoides* ointment on the average area (cm²) of the wounds of non-diabetic rats treated with *Ageratum conyzoides* ointment. n=4, *p<0.05; **p<0.01: significant differences from control lots (untreated). DTN: untreated diabetics; DTV: Vaseline-treated diabetics;Dtcicat: Cicatryl® -treated diabetics (reference product); DA. c (4 and 8 g): diabetics treated with Ageratumconyzoides ointment at 4 and 8 g/kg.

Effect of aqueous extract *Ageratum conyzoides* on quantification of Tnfα.

The figure 9 shows changes in levels of Tnfα in normal and diabetic rats treated with differents products, as a function of time. We find that in normal non-diabetic rats, the level of Tnfα decreases with variations of 9,730 pg/ml and 6,391 pg/ml, respectively, in rats treated with ointment at doses 4 and 8 g/kg at day 12. Wound scarring is almost complete in the 18th century. In contrast, in rats with diabetes, thereis a very high rate of Tnfα to J0. This rate gradually decreases after treatment with the various applied products and varies from: 15,712; 83,826 and 158,284 pg/ml at day 18 in rats treated with Cicatryl®, *Ageratum conyzoides* ointment at 4 and 8 g/kg. This decrease is significant ($p < 0.01$) for rats treated with *Ageratum conyzoides* ointment at 4 g to J_{18}, versus those treated with Cicatryl® at J_{12} and J_{18} ($p < 0,01$ and $p < 0,001$). For untreated and vaseline-treated diabetic rats, there was a steady increase in Tnfα from J_0 to J_{18}.

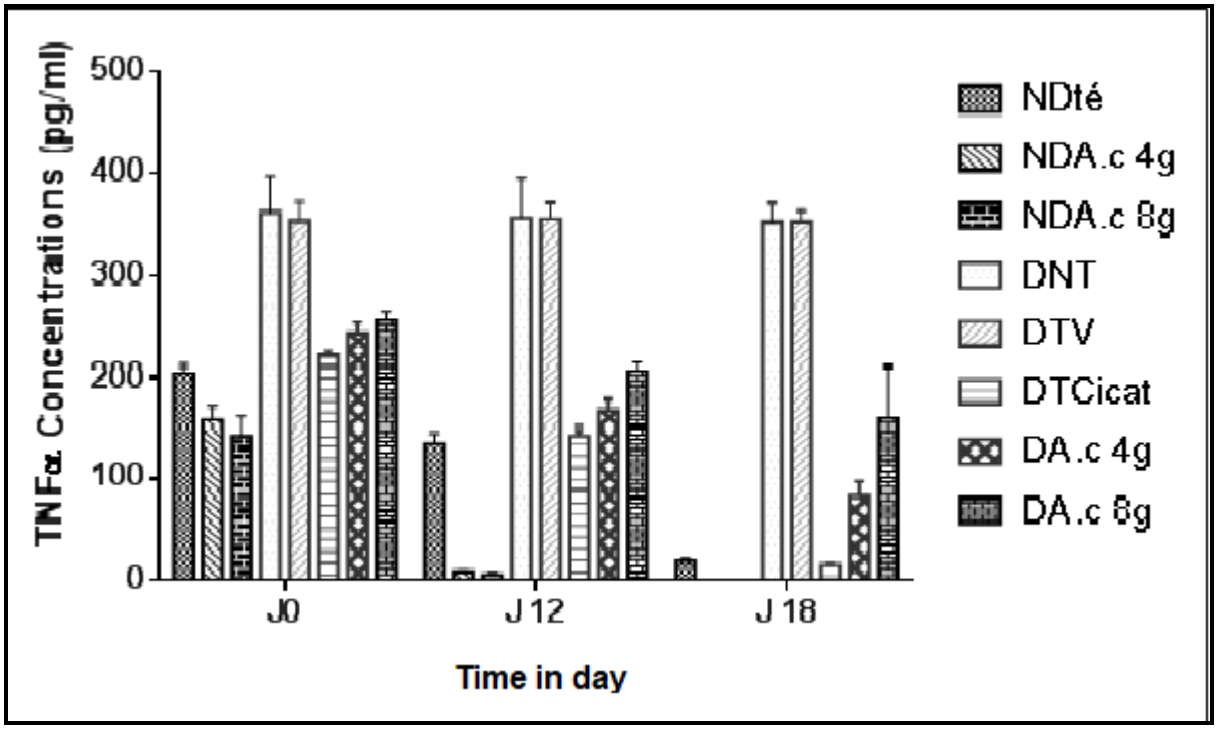


Figure 9: Changes in levels of Tnfα (pg/ml) in normal and diabetic rats compared to the differentproductsapplied to the wound over time. n =4; **p <0.01; ***p< 0.001: significantdifferencesfrom non-diabetic rats. Dte:untreateddiabetic (control); ND A.c (4 and 8 g): diabetictreatedwith*Ageratum conyzoides*ointment at 4 and 8 g; DTN: untreateddiabetic; DTV: diabetictreatedwith vaseline; Dtcicat: diabetictreatedwithCicatryl® (referenceproduct); DA. c (4 and 8 g): diabeticstreatedwith*Ageratum conyzoides*ointment at 4 and 8 g/kg.

Tests on organoleptic and physico chemical controls have shown that the ointment of *Ageratum conyzoides* is of homogeneous brown coffee color, creamy with pasty consistency, the bitter taste that would be attributed to the presence of alkaloids and tannins revealed by the phytochemical study of this plant, as well as mineral elements and other dissolved substances.[23] Indeed, *Ageratum conyzoides* ointment is not toxic in acute administration.[24] However, its primary skin irritation index allows to say that this ointment is non-irritating in topical application. But may be slightly so in administration eye at the conjunctiva level. Because, it can cause profuse watering and redness of the eye. This means that the plant would have compounds in its phytochemical composition that would be irritating in eye use. In addition, we note that the ointment of the aqueous extract of *Ageratum conyzoides* at doses of 4 and 8 g/kg accelerates the healing of the burn wound and the contraction of the cutaneous banks compared to the controls. The rate of contraction of a wound characterizes the closure of the wound, and is a function of its depth as well as the nutritional state of the subject. In our study, the rate of contraction was significant on day 16 in the batches of rats treated with *A.conyzoides* ointment and the combination of Silver Sulfadiazine and Chitosine Chlorydrate (Curapel®). This confirms, the healing effect of *Ageratum conyzoides* on burn wounds, as reported by: Brasil, (1989) and Durodola, (1977).[25,6]

According to data from the literature, some authors claim that: natural healing products exert their effects as a result of their antimicrobial, anti-inflammatory, antioxidant, stimulation of collagen synthesis and cell proliferation, then by their angiogenic effect.[26] Similarly, flavonoids and terpenes, which act as anti-inflammatories, analgesics, antioxidants, antimicrobials, anti allergic substances, are often cytokine modulators, and may therefore be of interest to cytokine control and

management. [27,28] Knowing that, pro-inflammatory cytokines TNF-α, IL-1β and IL-6, as well as others are accurate indicators of inflammation, which is a step in the healing process. [29] One can attribute the protective and barrier effect shown by the aqueous extract of *Ageratum conyzoides* on the wounds, to the various phytochemical constituents enteringits composition and which would block the action of these pro inflammatory cytokines, in favor of a cutaneous reepithelialisation.In addition, itis important to note that during our study, we found that rats treated with *Ageratum conyzoides* ointment had a clean scarwith a normal skin texture. While, those treated with the combination of Sulfadiazine Silver + Chitosine Chlorydrate showed erythoco eczematous epidermis. This implies that silver sulfadiazine, although a reference molecule used in the treatment of burnswith a broad spectrum of activity, has several side effects (cases of renaltoxicity and leukopenia). This limitsits long-term use on overly extensive wounds and delays their recovery, following allergic reactions to silver, which limit its use in some patients.[30,31,32] Therefore, the ointment of *Ageratum conyzoides* would be a product to promote by its non-aggressive effects of the skin and to orient towards the implementation improved phytomedicine. Regarding, the effect of ointment of aqueous extract of *Ageratum conyzoides* on diabetic wounds. The results showed that this plant not only caused a gradual decrease in the weight of the experimental animal, but also maintained its hyperglycemic state. Nevertheless, the ointment of *Ageratum conyzoides* at the studied doses (4 and 8g/kg) would have a slow healing power on diabetic wounds than on normal wounds. Indeed, all these observations would be explained by the factthat, the absence of the production of insulin due to the destruction of Beta cells has as a consequence, a non penetration of glucose into the cells from which hyperglycemia. In this case, these cells must find another biological substrate to produce energy and try to survive. This is how, fatty acids and aminoacids are used as the main source of energy, which could explain the considerable weightloss in the animal and the non-use of glucose by the target cells, and its storage in the liver. Leading to this hyperglycemia in diabetic rats.[33] Our work is consistent with that of: Jain et al., (2010) and Belayel and Berbache, (2005), who observed hyperglycemia in diabetic rats during treatment by local application of *Centaurea acaulis* and *Argania spinosaoils*.[34,21]

In fact, Belayel and Berbache, (2005), state that, under normal conditions, the evolution to the inflammatory, proliferative and remodeling phases is generally linear and synchronous throughout the wound bed.[21] However, the data obtained leads us to believe that diabetes alters this timing and the different regions of the wound are not at the same stage of healing. In addition, the dysfunctionis in the proliferative phase, with fibronectin and fibrin deposits that prevent cell remodeling and regeneration.[35] If diabetes seems to block the scar process at the proliferative level, *Ageratum conyzoides* seems to interfere with the inflammatory process, but weakly on the tissue phase processes in diabetics. In addition, the 4 g/kg dose of *Ageratum conyzoides* ointment appears to have more significant effects than the 8 g dose, suggesting that in the case of diabetes, this plant would have beneficial effects at low doses, as Ouattara–Soro et al., (2015) which showed that *Ageratum conyzoides* was low-dose hypoglycemic and high-dose hyperglycemic.[11] However, according to Lin et al., (2012), diabetic foot ulcers, at a 50% reduction before the 4th Wound week could prove a form of healing.[36] This probably explains the slowness shown by the ointment of the aqueous extract of *Ageratum conyzoides* to contract the diameters of diabetic wounds. In contrast, diabetic wounds treated with Cicatryl® showed a more significant decrease than those treated with the plant extract. This, because, this productis composed of Allantoine, Guaiazulene, Para-chlorometacresol and Alpha-tocopherolacetate, which would be compounds promoting an acceleration of the wound closure speed, while ensuring better epithelialization and production of collagen fibres.[36] In perspective, it would be interesting to continue in the long term, this part of the work on diabetic wounds. In order to confirm the action of *Ageratum conyzoides* on the healing process of skin wounds, we dosed Tnfα. These results show that *Ageratum conyzoides* inhibits the increased release of Tnfα during the inflammatory phase, stimulates growth factors and cells that promote healing. But the literature exploits the issue of diabetic wounds in sever always none of themsay:Pro-inflammatory cytokines may de lay wound healing, failing epithelialization, by inhibition of angiogenesis and decreased cell proliferation.[37] Interleukin-1β (IL-1β) and Tnfα participate in the recruitment of inflammatorycells by inducing the adhesion of leukocytes to the endothelium, maintenance of the mechanisms involved in inflammatory disorders, acne and chronic inflammation and increase the synthesis of prostacyclin and FAP. [38, 39] While, others complete saying that: In humans, the healing of diabetic wounds is characterized by a disturbed inflammatory process with an accumulation of pro-inflammatory cytokines, proteases and a decline in certain growth factors, that interfere with the healing process.[40,41,42,43,44] Diabetes induces both a decrease in these factors and an increase in TGF-β3, which blocks the migration of dermal cells by inhibiting diapedesis, while causing a decrease in activity of thesecells on the area to behealed.[45,46,47]

In addition, Trinh et al. (2011) evaluated the effect of α-terpineol, a monoterpene found in *Ageratum conyzoides* essential oil. [48] It has been shown that this monoterpene alcoholis capable of inhibiting pro-inflammatory cytokine expressions, modulating the production of TNF-α, by theirlipophilic character promoting absorption and the irrapid action, then increases the expression of the anti-inflammatory cytokine IL-10.[49,50,51,52,53,54,55]

From all the above, it can be said that the ointment of the aqueous extract of *Ageratum conyzoides* Linné has analgesic, anti inflammatory, modulating, stimulating and healing properties, favored by the contribution of its phyto compounds.

Finally, our results corroborate those obtained by: D'Alessio and al., (2014); Hansen and al., (2016); Rehman and al., (2014); Oliveira and al., (2016); Faqueti and al., (2016); Kim and al., (2014).[56,57,58,59,60]

CONCLUSION

This research work has enabled us to qualitatively characterize and evaluate the effect of the ointment of the aqueous extract of *Ageratum conyzoides* leaves on burn wounds and diabetic wounds induced in the Wistar rat. We consider that the ointment based on the aqueous extract of *Ageratum conyzoides* is brown, slightly bitter with a low acidic pH emitting a less strong or pungent smell. The ointment of *Ageratum conyzoides*is not irritating in topical application, but in contact with the eyes should be done with caution. In terms of its healing effect. The results showed that the ointment of Ageratum conyzoides significantly reduces the cutaneous banks of burn wounds and those of diabetics and promotes the rapid peel of the crust. This explains the shortening of the average duration of the complete reepithelialisation of the wounds. Thus, it can be assumed that the aqueous extract of the leaves of *Ageratum conyzoides* is a plant with healing potential useful for the health well-being of the populations.

CONFLICT OF INTEREST

Authors declare no conflict of interests.

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